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www.clinicalnutritionexperimental.com](http://www.clinicalnutritionexperimental.com)Maternal adiposity and maternal and cord blood concentrations of vitamin D [25(OH)D₃]Fernanda F.A. Simões^a, Natália P. Castro^a, Verônica V. Euclides^b,
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SUMMARY

Obesity is associated with lower concentrations of vitamin D [25(OH)D₃] in children, adolescents and adults, but it remains unclear whether maternal adiposity influences maternal and foetal concentrations of this vitamin. The objective of this cross-sectional study was to assess the relationship between maternal adiposity and maternal and cord blood concentrations of vitamin D. It involved 101 mother–newborn pairs from a public maternity in São Paulo city, Brazil. Demographic, socioeconomic and obstetric data, as well as anthropometry, physical activity and vitamin D supplementation during pregnancy, were investigated. Maternal adiposity was assessed by bioelectrical impedance. Maternal and cord blood concentrations of vitamin D were measured by high-performance liquid chromatography. Two multiple linear regression models that included maternal and cord blood vitamin D concentrations as outcomes and maternal adiposity as independent variable were used. No association was observed between maternal adiposity and maternal or cord blood concentrations of vitamin D. Maternal vitamin D concentration was associated with race, physical activity and vitamin D supplementation (adj. R² = 0.74). Cord blood vitamin D concentration was associated with maternal vitamin D concentration (adj. R² = 0.24). Although fat mass quantification is important to understand vitamin D status during all stages of life, this may not be true in pregnancy as race, vitamin D supplementation and physical activity appeared to

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be more relevant to vitamin D status. Understanding vitamin D metabolism in pregnancy may elucidate how or if adiposity influences maternal vitamin D status and how it impacts vitamin D transport to the foetus.

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1. Introduction

In recent years, there has been growing interest in vitamin D [25(OH)D₃] because of the global high prevalence of vitamin D insufficiency and deficiency, and increasing evidence associating deficits of this vitamin with negative health outcomes [1]. Low concentrations of vitamin D may lead to hypocalcaemic seizures, dilated cardiomyopathy, skeletal myopathy, congenital and infantile rickets, and osteomalacia. In addition to the importance of vitamin D for calcium and phosphorus homeostasis during bone growth, mineralization and neuromuscular activity [2], low concentrations of this vitamin have also been associated with cancer [3], cardiovascular diseases [4], diabetes and metabolic syndrome [5], lung disorders [6], and pregnancy complications [7].

The inverse association between vitamin D concentrations and obesity in children, adolescents and adults is well documented [8–10]. Adipose tissue is known to sequester vitamin D, consequently reducing its plasma concentrations and interfering with its bioavailability [11,12]. However, only few studies have explored vitamin D in pregnant women and their newborns. In a study involving 398 pregnant women from Pennsylvania, United States, an inverse relationship was observed between maternal obesity (body mass index, BMI ≥ 30) and maternal and blood concentrations of vitamin D [13]; however, subsequent studies did not confirm this finding. A study conducted in Chicago, United States, on 61 mother–newborn pairs [14] showed that maternal obesity was not associated with vitamin D concentration in the third trimester of pregnancy. However, vitamin D supplementation was not investigated during pregnancy. In a prospective study involving 105 pregnant Swedish women [15], obese women had lower concentrations of vitamin D in the first trimester, but there was no statistically significant difference in vitamin D concentrations between obese and eutrophic women in the second and third trimesters of pregnancy.

Previous studies have shown that cord blood concentration of vitamin D is positively associated with maternal concentrations of this vitamin [16,17], but there is speculation whether or not maternal obesity can impair placental transport of vitamin D. Josefson et al. [14] demonstrated that, irrespective of maternal vitamin D concentration, obese women transferred less vitamins to their foetus than eutrophic women. However, a recent prospective study involving 202 pregnant women found no association between maternal BMI and cord blood concentration of vitamin D [17].

There are controversial and insufficient data to confirm that maternal obesity/adiposity reduces maternal and/or newborn vitamin D concentrations. To our knowledge, no study has evaluated the effect of maternal adiposity per se, and not only of BMI, on cord blood vitamin D concentration. Therefore, the objective of this study was to assess the relationship between maternal adiposity and maternal and cord blood concentrations of vitamin D.

2. Materials and methods

2.1. Study design and participants

In this cross-sectional study, 101 women and their newborns apparently healthy were selected consecutively between April and June 2013 from a large public maternity unit in São Paulo city, Brazil, through their detailed hospital records and antenatal cards. Exclusion criteria were adolescence, multiple pregnancy, diabetes, hypertension, hormonal disorders, chronic infectious

diseases, drug or alcohol consumption, preterm delivery, low birth weight, and genetic disorders of the newborn.

2.2. Data collection

Shortly after delivery, the women were invited to participate in the study and were asked about demographic, socioeconomic and obstetric factors. A socioeconomic questionnaire was applied, assigning scores to maternal education and housing conditions (television sets, CD players, vehicles, washing machines, DVD players, refrigerators/freezers, and bathrooms). The following obstetric data were collected from the mothers: pre-pregnancy BMI, maternal date of birth, maternal weight in all antenatal visits, newborn date and time of birth, type of delivery, sex, gestational age (GA), birth weight, and length. The data were verified against antenatal cards and hospital records.

Gestational age was determined by a combination of ultrasonography performed up to week 20 of gestation, the Capurro method [18] determined within 12–48 h after delivery, and the date of the last menstrual period. When there was a discrepancy of one week or less between at least two of the GA determinations assessed by the three different methods, one of them was chosen, giving preference to the order of the methods cited above.

Maternal physical activity was investigated using the long form of the International Physical Activity Questionnaire (IPAQ), validated for the Brazilian population [19]. The data were analysed according to the Guidelines for Data Processing and Analysis of the IPAQ [20].

2.3. Anthropometry and body composition of the mothers and newborns

Maternal height was measured with a Tonelli stadiometer (120A, Criciúma, Santa Catarina, Brazil). Maternal weight and adiposity were evaluated with a bioelectrical impedance analyser (Inbody370, Biospace®, Gangnam-Gu, Seoul, Korea). The nutritional status of the mothers was determined based on BMI according to the WHO classification: underweight: $< 18.5 \text{ kg/m}^2$; normal weight: $18.5\text{--}24.99 \text{ kg/m}^2$; overweight: $25.0\text{--}29.99 \text{ kg/m}^2$; obese: $\geq 30.0 \text{ kg/m}^2$. Maternal adiposity was assessed by fat mass (kg) and fat mass percentage.

Newborn length was measured with a Seca® infantometer (416, Hamburg, Hamburg, Germany) to the nearest 0.1 cm. Weight (to the nearest 1 g) and body composition were assessed by air displacement plethysmography (PEA POD®, Cosmed, San Francisco, CA, USA). All measurements were performed 24–72 h after birth.

2.4. Vitamin D [25(OH)D₃]

Fasting cord and maternal blood samples were collected into EDTA vacutainers (Becton Dickinson, Rutherford, NJ, USA) up to 10 min and 24–72 h after delivery, respectively. The samples were centrifuged for 15 min at 960 g (Fanem®, Excelsa II 206 BL, São Paulo, SP, Brazil) and plasma was stored at -70°C until the time of analysis. Vitamin D was extracted from plasma (500 μL) by the addition of 25 μL HPLC ethanol solution (Merck, Hessen, Darmstadt, Germany) and incubation for 10 min at room temperature. After this period, 500 μL of the mobile phase (methanol:isopropanol, 98:2, v/v) (Merck, Hessen, Darmstadt, Germany) was added and the mixture was vortexed for 15 s (Biomixer MVS-1, Jersey city, NJ, USA). One milliliter hexane (Merck, Hessen, Darmstadt, Germany) was added and the final solution was mixed (60 s). The solution was centrifuged (Eppendorf® 5415C, Hamburg, Hamburg, Germany) for 4 min at 960 g and the supernatant (250 μL) was transferred to a clean test tube. This procedure was repeated twice and evaporated for 10 min at room temperature in a Centrivap concentrator (Labconco, Kansas City, MO, USA). The extracted vitamin was reconstituted in 250 μL methanol, submitted to ultrasonic agitation for 5 min, and filtered through polypropylene membranes (0.45 μM). Analysis was performed in an LC-20AT HPLC system (Shimadzu, Inc., Chiyoda-Ku, Tokyo, Japan), equipped with an SIL-20AC automatic injector, CBM-20A controller, CTO-20A column oven (40°C), and SPD-M20A diode array detector, using a Luna C18 separation column ($250.0 \times 4.6 \text{ mm}$, 5- μm particle size; Phenomenex, Torrance, CA, USA). The flow rate of the mobile phase was 0.8 mL/min for 15 min. The retention time for 25(OH)D₃ was 8.5 min and the injection volume was 100 μL . A 5-

point external calibration curve was used for 25(OH)D₃ analysis and the extraction efficiency was 96.15%.

The concentrations of 25(OH)D₃ were determined according to Neyestani et al. [21] and blood samples were collected during the same season to avoid differences in vitamin concentrations. The reference values used to define vitamin D insufficiency and deficiency in mothers and newborns were <20 ng/mL (50 nmol/L) and 21–29 ng/mL (52.5–72.5 nmol/L), respectively [22].

2.5. Ethical aspects

The study was approved by the Ethics Committees of the School of Public Health, University of São Paulo, and of the Dr. Mário de Moraes Altenfelder Silva Maternity Unit. All mothers gave written informed consent for themselves and their newborns.

2.6. Statistical analysis

Data were analysed using the Statistical Package for the Social Science for Windows® (version 20.0; SPSS, Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to determine whether the outcome variables (maternal and cord blood vitamin D) followed a normal distribution. Since no normal distribution was observed, the data were log transformed. Variance homogeneity was verified by the Levene test. Two multivariate linear regression models were developed to evaluate the associations between maternal adiposity and maternal and cord blood concentrations of vitamin D using backward stepwise selection.

The relationship of maternal vitamin D concentration (outcome) with the independent variables - maternal adiposity and confounders (maternal age, race, physical activity, parity, height, pre-pregnancy BMI, weight gain, and vitamin D supplementation in pregnancy) was determined by univariate linear regression. Variables with $p \leq 0.20$ in the univariate analysis were selected for entry into the first multivariate linear regression model.

The relationship of cord blood vitamin D concentration (outcome) with the independent variables - maternal adiposity and confounders (maternal age, race, physical activity, parity, height, pre-pregnancy BMI, weight gain, vitamin D supplementation in pregnancy, maternal vitamin D concentration, GA, and newborn sex, weight, length and fat mass percentage) was determined by univariate linear regression. Variables with $p \leq 0.20$ in the univariate analysis were selected for entry into the second multivariate linear regression model.

A p value ≤ 0.05 was considered significant.

3. Results

Table 1 shows the characteristics of the 101 mother–newborn pairs included in the study. The mean maternal age (SD) was 25.81 (5.15) years and 58.4% of the mothers were black or mulatto. Most women (69.3%) were classified as belonging to socioeconomic stratum “C” (middle class in Brazil), with a mean (SD) monthly *per capita* income of R\$ 608.73 (approximately US\$ 196.36). Almost half of the women (42.6%) had low levels of physical activity, with an average time spent sitting of 4.25 h on weekdays and of 3.59 h on weekends. Most women were multiparous (65.3%) and overweight or obese (69.3%) at the beginning of pregnancy. The mean (SD) weight gain during pregnancy was 14.3 (5.39) kg, and mean (SD) fat mass and fat mass percentage were 24.26 (10.53) kg and 32.32% (7.74), respectively. Almost 9% of the women used vitamin D supplementation during pregnancy. Mean (SD) concentrations of maternal and cord vitamin D were 30.86 (21.17) ng/mL and 10.75 (6.95) ng/mL, respectively.

More than half of the newborns (51.5%) were female, with a mean GA (SD) of 39.65 (1.37) weeks, birth weight of 3322.46 (458.4) g, length of 48.71 (2.04) cm, fat mass of 0.27 (0.16) kg, and fat mass percentage of 8.55 (4.37).

Figure 1 shows the distribution of maternal and cord blood concentrations of vitamin D on a logarithmic scale. Vitamin D insufficiency and deficiency were 15.4% and 41.4% in mothers, respectively, and 7% and 88.4% in newborns (Fig. 2).

Table 1

Maternal and newborn characteristics (n = 101).

Maternal characteristics	N	%	Mean (SD)
Age (years)	101	100	25.81 (5.15)
Race			
Black	17	16.8	
Mulatto	42	41.6	
White	41	40.6	
Unknown	1	1.0	
Per capita monthly income (R\$)*	70	69.3	608.73 (434.91)
Level of physical activity			
Low	43	42.6	
Moderate	35	34.7	
High	11	10.9	
Unknown	12	11.9	
Parity			
Primiparous	35	34.7	
Multiparous	66	65.3	
Height (cm)	98	97.0	159.94 (6.86)
Pre-pregnancy BMI (Kg/m ²)	99	98.0	27.79 (5.61)
<18.50 (underweight)	3	3.0	
18.50–24.99 (normal range)	26	25.7	
25.00–29.99 (overweight)	42	41.6	
≥30.00 (obese)	28	27.7	
Unknown	2	2.0	
Weight gain in pregnancy (Kg)	81	80.2	14.30 (5.39)
Fat mass (Kg)	99	98.0	24.26 (10.53)
Fat mass (%)	99	98.0	32.32 (7.74)
Vitamin D concentration (ng/mL)	99	98.0	30.65 (21.17)
Vitamin D supplementation			
No	92	91.1	
Yes	9	8.9	
Newborn's characteristics			
Sex			
Male	49	48.5	
Female	52	51.5	
Gestational age (weeks)	101		39.65 (1.37)
Birth weight (g)	101		3322.46 (458.40)
Length at birth (cm)	97		48.71 (2.04)
Fat mass (Kg)	101		0.27 (0.16)
Fat mass (%)	101		8.55 (4.37)
Cord vitamin D concentration (ng/mL)	86		10.75 (6.95)

*US\$1.00 = R\$2.14.

Univariate linear regression analysis revealed no associations between maternal adiposity and maternal ($p = 0.55$) or cord blood ($p = 0.79$) concentrations of vitamin D. Race ($p = 0.017$), physical activity ($p = 0.009$) and vitamin D supplementation ($p = 0.02$) were associated with maternal vitamin D concentration (adj. $R^2 = 0.737$) in the multivariate regression model, considering maternal vitamin D concentration as outcome (Table 2).

Univariate linear regression analysis showed an association between cord blood and maternal concentrations of vitamin D ($p = 0.019$). The relationship remained significant after adjustment for confounding variables in the multivariate regression model (adj. $R^2 = 0.235$), considering cord blood vitamin D concentration as outcome (Table 3).

4. Discussion

This study found no associations between maternal adiposity and maternal or cord blood concentrations of vitamin D. An association between maternal obesity and vitamin D concentration has

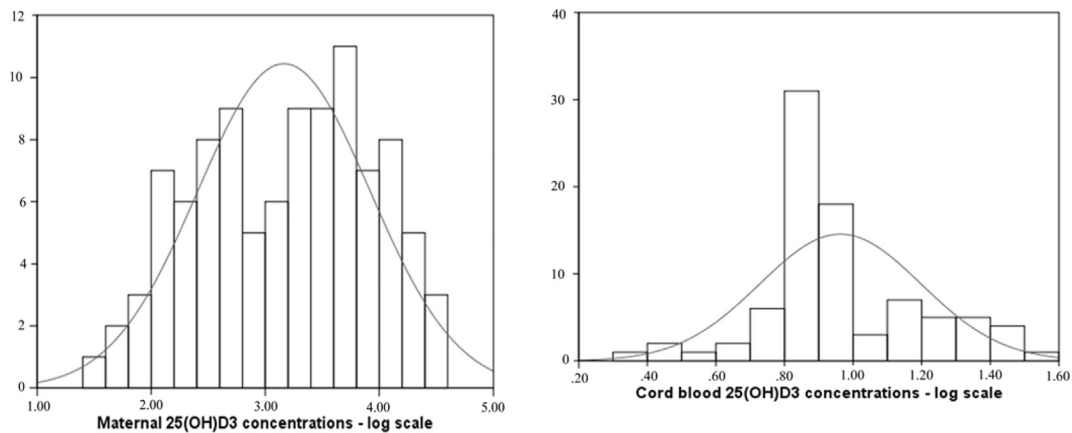


Figure 1. Distribution of maternal (n = 99) and cord blood (n = 86) concentrations of vitamin D on a logarithmic scale.

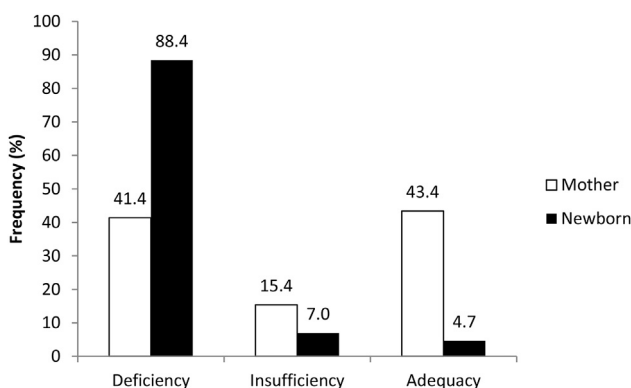


Figure 2. Distribution (%) of mothers (n = 99) and newborns (n = 86) according to their vitamin D status.

been suggested in a previous cohort study [13]. The authors observed a negative relationship between pre-pregnancy BMI and vitamin D concentration in pregnant women at 4–22 week gestation and at term after controlling for race, GA, vitamin D supplementation, physical activity before pregnancy, and maternal age. Similar to other cross-sectional [14,22] and longitudinal studies [15,17], our results did not show a relationship between maternal obesity and vitamin D concentration. However, only one of those studies investigated adiposity and was not limited to pre-pregnancy BMI [15]. Since the reduction of plasma vitamin D concentrations in obese individuals is explained primarily by the sequestration of this vitamin by adipose tissue [11,12], it is likely that fat mass quantification is fundamental to understand this relationship in future studies.

Possible explanations for the lack of an association between adiposity and maternal vitamin D concentration are: 1) changes in vitamin D metabolism during pregnancy [22,23], 2) the lack of determination of vitamin D protein ligands [24], and 3) increased total body water content (haemodilution) observed during pregnancy. These mechanisms remain to be elucidated.

In this study, maternal vitamin D concentration was associated with race, vitamin D supplementation and physical activity in pregnancy. Race is known to have an independent and important impact on blood vitamin D concentration [25]. As suggested by Karlsson et al. [15], this variable should always be documented in studies involving maternal–foetal vitamin D status since it can cause misinterpretation of the data. White women exhibited an increase in log transformed vitamin D concentration of 0.116 when compared to mulatto or black women. Nevertheless, this variable did not interfere with cord vitamin D concentration.

Almost 9% of the women reported vitamin D supplementation during pregnancy. As in Brazil, vitamin D supplementation is not part of the antenatal care protocol in other countries. However, in this study, vitamin D supplementation was the variable that had the largest impact on log transformed maternal vitamin D concentration, contributing to an increase of 0.614 in the vitamin. Despite the lack of studies investigating vitamin D status in pregnant Brazilian women, we are encouraged to reflect about the need and benefits of implementing vitamin D supplementation as part of the antenatal care protocol in view of the high prevalence of vitamin D insufficiency/deficiency in the Brazilian population [26,27].

Physical activity during pregnancy, which was low in most of the women participating in the study (42.6%), can have a protective effect against vitamin D deficiency. In cases of outdoor physical activities, this effect has been attributed to sun exposure and to the season of the year when the blood sample was collected [28]. In the present study, blood was collected during the same season of the year (autumn), but we did not determine whether physical activity was performed outdoors or indoors. Since walking is the most common physical activity performed by Brazilian adults, we believe that part of the participants walked outdoors, generally in public parks where sunlight is readily available in autumn. This fact would explain the association between physical activity and maternal vitamin D concentration. Based on the results of this study it is important to implement interventions to increase outdoor physical activity in Brazil.

Table 2
Multivariate linear regression model considering log transformed maternal vitamin D concentration as outcome and maternal adiposity as independent variable.

Variables	β	CI (95%)	P	Adj. R ²
Race	0.116	0.021–0.211	0.017	0.737
Level of physical activity	0.290	0.074–0.051	0.009	
Vitamin D supplementation	0.614	0.097–1.131	0.020	
Constant	2.628	2.226–2.230	<0.001	

Adjusted for the following confounders: maternal age, race, physical activity, parity, height, pre-pregnancy BMI, weight gain and vitamin D supplementation in pregnancy.

Table 3
Multivariate linear regression model, considering log transformed cord vitamin D concentration as outcome and maternal adiposity as independent variable.

Variable	β	CI (95%)	P	Adj. R ²
Maternal vitamin D concentration	0.078	0.013–0.143	0.019	0.235
Constant	0.719	0.506–0.931	<0.001	

Adjusted for the following confounders: maternal age, race, physical activity, parity, height, pre-pregnancy BMI, weight gain and vitamin D supplementation in pregnancy, maternal vitamin D concentration, GA, and newborn's sex, weight, length and fat mass percentage.

It is known that neonatal vitamin D concentration relies on maternal vitamin D status and the ability of the placenta to transport this vitamin [14]. However, there is no evidence that obesity or maternal adiposity impairs vitamin D transport through the placenta. To our knowledge, this is the first study that evaluated the relationship between maternal adiposity and cord blood vitamin D concentration, reporting no association between these variables. In addition, no association was found between pre-pregnancy BMI and cord blood vitamin D concentration.

It is important to note that conditions that affect maternal vitamin D transport to the foetus, such as drug, tobacco and alcohol use, hyperglycaemia, hypertension, preterm/post-term deliveries and adolescent pregnancy, were excluded from the study. One may speculate that maternal adiposity/obesity per se does not interfere with maternal vitamin D transport to the foetus in the absence of other associated conditions.

Bioelectrical impedance, the method used in this study to assess maternal body composition, has limitations when applied during pregnancy and shortly after delivery because of an altered relationship between intracellular and extracellular water and higher water content in the trunk compared to other body segments [29]. However, Karlsson et al. [15] obtained similar results using air displacement plethysmography to estimate maternal body composition, indicating that maternal adiposity may not interfere with maternal or cord blood vitamin D concentration during pregnancy, regardless of the method used.

Inadequate vitamin D intake during pregnancy is frequently reported in cross-sectional and longitudinal studies [30] and has been implicated in low maternal and cord blood vitamin D concentrations. Prenatal nutritional counselling is important to stimulate adequate vitamin D consumption, by increasing the intake of vitamin D-rich foods such as fatty fish, liver, eggs, vegetables and fortified dairy products.

In conclusion, this study shows that maternal adiposity was not associated with maternal or cord blood concentration of vitamin D. However, maternal vitamin D concentration was positively associated with cord vitamin D concentration, confirming the results of previous studies.

Although fat mass quantification is important to understand vitamin D status during all stages of life, this might not be true in pregnancy as race, vitamin D supplementation and physical activity appeared to be more relevant to vitamin D status. Understanding how vitamin D metabolism changes during pregnancy may shed light on how or if adiposity influences vitamin D status in pregnancy. In addition, it would be relevant to explore in a further study vitamin D-binding protein concentrations and genetic polymorphisms in vitamin D receptor.

Statement of authorship

PHCR and FAS conceived and designed the study. FAS, NPC, VVE, and LAL collected the data. AAP was responsible for data analysis. All authors were involved in the interpretation of the data and drafting of the paper, critically reviewed its content, and approved the final version submitted for publication.

Conflict of interest statement

The authors declare no conflict of interest.

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